Journal of Forensic and Legal Medicine 18 (2011) 191–197

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Review

# DNA Profiling and forensic dentistry – A review of the recent concepts and trends

B.C. Manjunath BDS, MDS, Professor [a,*], B.R. Chandrashekar BDS, MDS, Reader [b], Melkundi Mahesh BDS, MDS, Assistant Professor [c], R.M. Vatchala Rani BDS, MDS, Post Graduate Student [d]

[a] Department of Public Health Dentistry, Jaipur Dental College, Dhand, Amer Tehsil, NH-8, Jaipur 303101, Rajasthan, India
[b] Department of Community Dentistry, Peoples Dental Academy, Bhanpur, Bhopal, India
[c] Department of Oral Pathology and Microbiology, College of Dental Sciences & Hospital, Rau, Indore, Madhya Pradesh, India
[d] Department of Oral Pathology and Microbiology, Jaipur Dental College, Jaipur, Rajasthan, India

ARTICLE INFO

*Article history:*
Received 20 August 2010
Accepted 20 February 2011
Available online 23 March 2011

*Keywords:*
DNA Profiling
Forensic dentistry
DNA isolation
Short tandem repeats
Short nucleotide polymorphism
mtDNA analysis

ABSTRACT

*Background:* Teeth are amongst the hardest structures of the human body which are resistant to adverse conditions such as incineration, immersion, trauma, mutilation, decomposition and hence, used in forensic investigations. It is also a valuable source of DNA as other parts of the body gets destroyed or degraded in mass disasters. The fast technological advancements in DNA research have revolutionized the field of forensic medicine and the present work was undertaken to provide an insight in to the recent concepts of DNA profiling in Forensic dentistry.
*Methods:* Articles were identified by searches in PubMed and Embase electronic databases from 1980 through July 2010.
*Results:* DNA profiling provides an exact identification of an individual in mass disasters, identification of culprits in crime scene investigations and solving paternity issues as well. It also provides information regarding the physical characteristics, ethnicity and sex determination.
*Conclusion:* Teeth should be considered for DNA analysis as they are rich sources of quality DNA which can be utilised in all forensic investigations. From variable number tandem repeats (VNTRs) to single nucleotide polymorphism (SNP), the field of forensic DNA research has been true to the characteristics of any scientific process and it has never been static but represents a continuous evolution of technological development.



## 1. Introduction

Forensic dentistry is an important sub specialty of forensic medicine which is contributing immensely in solving difficult criminal cases and in identification of individuals in mass disasters. The world has experienced many mass disasters like acts of terrorism, bombings, earthquakes, hurricanes, tsunami, railway accidents, air crashes and other transportation mishaps in the recent times.[1] In these circumstances, exact identification of an individual becomes difficult as the bodies are charred or destroyed beyond visual recognition. Dental identifications have always played a key role in natural and man made disaster situations as the teeth and jaws resist extreme temperature conditions and can provide a valuable clue in identification of an individual. Disaster victim identification traditionally relies on the combined efforts of police, dentists and pathologists where ante mortem information from the missing persons are compared with post mortem data of the dead persons.[2] In some of the cases, dental investigations may fail due to lack of proper ante-mortem records.[3] The dental records such as casts, models, radiographs will be very valuable for comparing with post mortem records in cases of mass casualties.[4,5]

If ante mortem data is unavailable then the exact identification becomes difficult and only the DNA profiling systems can reveal the exact identity of a person. Because of the resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation and decomposition, teeth represent an excellent source of DNA material.[6,7] When the conventional dental identification methods fail, this biological material can provide the necessary link to prove identity.[8,9] Matching of the DNA extracted from the teeth of an unidentified individual with DNA isolated from known ante mortem samples such as stored blood, tooth brush, hairbrush, clothing, cervical smear, biopsy, to a parent or sibling is the usual procedure in DNA analysis.[10]

* Corresponding author. Tel.: +91 1426 234175, +91 9413622653 (mobile); fax: +91 1426 234176.
*E-mail addresses:* bcmanju10@rediffmail.com (B.C. Manjunath), drchandrubr@yahoo.com (B.R. Chandrashekar), maheshop@gmail.com (M. Mahesh), drvatchu@yahoo.com (R.M. Vatchala Rani).

1752-928X/$ – see front matter 
doi:10.1016/j.jflm.2011.02.005

The fast technological development in molecular biology, forensic applications of human polymorphism and the increasing knowledge of the human genome is having a major impact on forensic medicine. Genetic characterization of individuals at the DNA level enables identity testing from a minimal amount of biological specimens.[11,12] DNA typing systems like Short tandem repeats (STRs), Single nucleotide polymorphism (SNPs), X and Y chromosome analysis have revolutionised the field of forensic medicine which is now referred as “Forensic Genetics”.[13] The DNA analysis can provide an exact identification of a person and also give details about an individual’s physical characteristics, ethnicity, place of origin, and sex. The currently available DNA tests have high reliability and are also accepted as legal proofs in courts.[14,15] This article provides a comprehensive review of the recent concepts regarding DNA extraction, isolation, amplification, and DNA profiling systems applicable to forensic dentistry.

## 2. Methodology

### 2.1. Search strategy

In this review, articles were identified by searches on electronic data bases such as Pub med database and EMBASE from 1980 through July 2010 in the English language. The following search terms were used: “DNA Profiling in forensic dentistry”, “DNA finger printing in forensic dentistry”, “Teeth and DNA analysis”, “Dental pulp and DNA analysis”, “DNA isolation methods”, “DNA amplification”, “Forensic DNA Typing”, “STR Typing” “mtDNA Typing”, “SNP typing”, “Y chromosome analysis”, “X chromosome analysis”.

### 2.2. Selection criteria

The cross sectional studies, cohort studies, randomised controlled trials, in vitro studies, which were describing the development, use, strategies and methods or comparison of DNA technologies applicable to forensic dentistry were included.

### 2.3. Data collection and analysis

The information was abstracted from each of the included studies. The DNA technologies and methods applicable to forensic dentistry were assessed and compared descriptively with respect to their usefulness, comprehensiveness, advantages, and disadvantages.

## 3. Role of DNA in identification

Any type of organism can be identified by examination of DNA sequences unique to that species. Every cell of an individual carries a copy of the DNA. Order of base pairs in the DNA of every individual is different except identical twins. The uniqueness is due to the intron regions of DNA which contain sequences that are 20–100 bp in length that are repeated at different locations (loci) along the chromosome like AGACTAGACATT– AGATTAGGCATT which are called sequence polymorphism. The length polymorphism like(-AATG)(AATG)(AATG) (3 repeats) and (AATG)(AATG) (2 repeats) are termed as Short tandem repeats (STRs) which are used in forensic identification.[16,17]

## 4. History

### 4.1. The discovery of DNA

DNA, the molecule that carries genetic information from one generation to the other was discovered by James Watson and Francis Crick in 1953.[18] They presented the structure of the DNA-helix for the first time and shared the Nobel Prize with Maurice Wilkins, nine years later in 1962, for solving one of the most important biological riddles.[19]

### 4.2. Forensic DNA analysis

It has been 25 years since ‘DNA fingerprinting’ or DNA typing (profiling) as it is now known, was first described in 1985 by an English geneticist named Dr. Alec Jeffreys.[20] He discovered that certain regions of DNA contained DNA sequences that were repeated and could differ in each individual.[21] Jeffreys developed a technique to examine the length variation of these DNA repeat sequences which had the ability to perform human identity tests.[22] These DNA repeat regions are called as VNTRs (Variable number tandem repeats) and was first used to solve an English immigration case and shortly thereafter to find out the culprit in a double homicide case and since then, human identification using DNA profiling methods has been widespread.[23]

“DNA finger printing” the analogy to fingerprinting coined by Alec Jeffreys, was found to be seriously unhelpful. Evett and Buckleton advocated a change to DNA profiling that has been largely accepted. DNA profiling made the system more sensitive, more reproducible, amenable to computer data basing, and soon became the standard forensic DNA system used in criminal case work as well as paternity testing worldwide.[24]

## 5. Teeth and DNA analysis

DNA is preserved in the teeth and bones for a very long period and thus are a valuable source of information. Ancient DNA (aDNA) analysis can be carried out through extraction of the tiny amounts of DNA remaining in samples that are hundreds to tens of thousands of year’s old.[25] Teeth are resistant to adverse conditions degrading the DNA, such as humidity, high temperature, and the microbial action.[26–28] In the tooth, dentin and pulp are a rich sources of DNA which can be successfully extracted.[28,29] Results of a study demonstrated that sufficient quantity of DNA can be extracted from the crown body, root body, and root tip. However the root body is the region which yields highest quantities of DNA.[30] Not only the quantity of DNA available for the laboratory is important, but also the quality and purity. Furthermore, an abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis.[31,32]

## 6. Sampling of the tooth for DNA extraction

Various methods have been reported regarding the extraction of DNA from the tooth which includes sectioning of teeth horizontally at the cemento- enamel junction or vertically up to root tip, scraping and aspiration.[33,34]

In a modified horizontal sectioning procedure, the tooth is circumferentially scored 1 mm below the cemento-enamel junction with a long-shanked round bur leaving a 2–3 mm wide isthmus of intact tooth structure on the facial surface. The crown is then manually separated from the root. A large round bur is then used to remove as much coronal and root dentin as possible. Restoring the shape of the tooth back to its pre-sampled state is possible, by re-approximating the crown and root portions of the tooth at the isthmus and can be returned to the surviving family members after completion of the analytical procedures and publishing of the results. This method has many advantages like simplicity, ease of access, preservation of crown as well as root structure, and the ability to restore the tooth very close to its pre-sampled state.[28]

DNA can also be extracted by horizontal sectioning of the tooth with extirpation of the dental pulp from the chamber and grinding the remaining root to a fine powder saving the crowns of the teeth. The dentine cement powder and dental pulp can be used for DNA extraction separately.[35]

Crushing of the teeth or cryogenic grinding is another method which also yields sufficient amount of the genetic material where a freezer mill is used to pulverise teeth under frozen preparation in liquid nitrogen under sterile conditions. The drawback of this technique is that it can result in the total destruction of the tooth sample.[8]

Recently conservative methods are gaining importance rather than grinding the whole tooth. Tooth samples of archaeological importance or museum samples have to be preserved. Since the dental pulp is also rich source of the genetic material, the pulp chamber can be accessed through conventional access cavity preparation and dental pulp can be retrieved. The advantages of this technique are its simplicity, relatively low cost and preservation of the tooth integrity which can be considered in forensic investigations.[33,36]

## 7. DNA isolation methods

Forensic DNA analysis can be increasingly problematic since samples from the scene of crime or a mass disaster may contain only minute amounts of DNA, which may include polymerase chain reaction (PCR)-inhibitors. Efficient DNA extraction procedures as well as accurate DNA quantification methods are critical steps involved in the process of successful DNA analysis of such samples.[37]

### *7.1. Organic extraction (phenol – chloroform method)*

The phenol-chloroform method is a sensitive but oldest method for the extraction of DNA from a wide variety of forensic samples. Even though it yields high quality DNA, it has many disadvantages like it is very laborious, time consuming, handling of dangerous organic solvents and can only be done if abundance of sample is available. Due to these setbacks, the phenol/chloroform method has been superceded by many other techniques which have made it irrelevant at present.[37,38]

### *7.2. Silica based DNA extraction methods*

Silica based methods are suitable for extraction of DNA from ancient bones and teeth (aDNA). The silica-based extraction method showed better results in nuclear STR typing from degraded bone samples than a commonly used phenol/chloroform method.[39] Digesting of the bone powders with proteinase K, and then extracting purified DNA directly using silica-based spin columns (QIAquick, QIAGEN) is also an efficient method of DNA isolation.[40] Recovery of PCR-amplifiable DNA from ancient bone and teeth specimens can be maximised by a combination of DNA extraction from bone powder using a buffer consisting solely of EDTA and proteinase K, and purification of the DNA by binding to silica in the presence of high concentrations of guanidinium thiocyanate. It also results in minimizing co-extraction of substances that inhibit PCR.[41] Silica-based aDNA extracts using ion-exchange columns considerably improved PCR amplification and can be useful in poorly preserved, PCR-resistant, ancient samples.[42] A combination of total demineralization and ion-exchange columns increases approximately three times higher DNA recovery from old bone compared to incomplete demineralization method.[43,44]

### *7.3. Chelex 100*

Procedures utilizing Chelex 100 chelating resin have been developed for extracting DNA from forensic-type samples to be used with the PCR. The procedures are simple, rapid, involve no organic solvents and do not require multiple tube transfers for most types of samples.[45] The extraction of DNA from dental pulp using this method is reported to be efficient compared to proteinase K and phenol-chloroform extraction. Chelex 100-based DNA extraction, amplification, and typing are possible in incinerated teeth.[46]

### *7.4. Commercial DNA extraction kits*

The PrepFiler Forensic DNA extraction kit enables isolation of genomic DNA from a variety of biological samples. The kit facilitates reversible binding of DNA with magnetic particles resulting in high DNA recovery from samples with very low and high quantities of biological materials such as saliva on swabs, semen stains on cotton fabric, samples exposed to environment, samples with polymerase chain reaction (PCR) inhibitors, blood stains on denim, cotton cloth, FTA paper, and touch evidence-type samples.[47,48]

## 8. DNA amplification methods

### *8.1. The polymerase chain reaction (PCR)*

The polymerase chain reaction (PCR) is a technique in molecular biology to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.[49] Polymerase Chain Reaction (PCR) was developed in the year 1983 by Kary Mullis for which he was awarded Nobel prize in chemistry in 1993.[50,51] The drawbacks of PCR is that Quantification is exceedingly difficult.[52]

### *8.2. Real time polymerase chain reaction*

Real time PCR is refinement of the old PCR technique and is the most powerful tool for DNA amplification. Some of the limitations of the old PCR were resolved in 1992 by the development of real-time PCR. It was developed by Higuchi et al.[53] Typical uses of real-time PCR includes pathogen detection, gene expression analysis, single nucleotide polymorphism (SNP) analysis, analysis of chromosome aberrations, and most recently the protein detection as well.[52,54,55]

### *8.3. AmpFlSTR MiniFiler and AmpFlSTR Identifiler PCR amplification kits*

The AmpFlSTR MiniFiler polymerase chain reaction amplification kit, developed and supplied by applied biosystems, compliments the AmpFlSTR identifiler polymerase chain reaction amplification kit by improving the success rate when profiling DNA that is degraded or contains inhibitors.[56]

## 9. DNA typing (profiling) systems

DNA profiling uses a variety of DNA typing systems including: Restriction Fragment Length Polymorphism (RFLP), Short Tandem Repeat (STR) typing, Mitochondrial DNA (mtDNA) analysis, Y chromosome analysis, X-chromosome STR Typing, Single Nucleotide Polymorphism (SNP) typing and Gender typing.

### 9.1. Restriction Fragment Length Polymorphism (RFLP) typing

RFLP is one of the oldest DNA typing methods which are no longer practiced at present. It is used for analyzing the variable lengths of DNA fragments that result from digesting a DNA sample with a special kind of restriction enzyme called "restriction endonuclease" which sections DNA at a specific sequence pattern known as a restriction endonuclease recognition site. With the development of newer, more efficient DNA-analysis techniques, RFLP is not used as it once was, because it requires relatively large amounts of DNA, cannot be performed with the samples degraded by environmental factors and also takes longer time to get the results.[57,58]

### 9.2. Short tandem repeats (STRs) typing

They are described as short stretches of DNA that are repeated at various locations throughout the human genome and this technology is used to evaluate specific regions (loci) within nuclear DNA. Each person has some STRs that were inherited from father and some from mother but however no person has STRs that are identical to those of either parent. The uniqueness of an individual's STRs provides the scientific marker of identity and hence is helpful in forensic identification and paternity testing. Since there are numerous variations in STRs the chance of matching two different individuals is very remote.[59–61]

#### 9.2.1. CODIS

CODIS stands for Combined DNA Index System. It was established and funded by the Federal Bureau of Investigation (FBI) which has become the core of the national DNA database. It was developed specifically for enabling public forensic DNA laboratories to create searchable DNA databases of authorized DNA profiles. The CODIS software permits laboratories throughout the United States (US) to share and compare DNA data. In addition, it provides a central database of the DNA profiles from all user laboratories. The odd that two individuals will have the same 13-loci DNA profile is about one in a billion. The 13 CODIS locations are TH01, TPOX, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11. The sex typing marker amelogenin is typically included in STR multiplexes that cover the 13 core STR loci. The United States maintains the largest DNA database in the world: The Combined DNA Index System, with over 60 million records as of 2007.[62–64]

#### 9.2.2. Uses of bones and teeth in STR analysis

STR can also be used for identification of bodies in the mass disasters and old skeletal remains.[65–67] DNA isolated from ancient skeletal remains can be subjected to STR analysis and even though the DNA present in these ancient remains appeared much degraded, it was better conserved in tooth than in bone samples.[68] Correct sampling of the body part is essential to obtain good quality DNA for analysis. Milos A et al[69] reported that the highest success rates were observed with samples from dense cortical bone of weight-bearing leg bones (femur 86.9%) and intact teeth also exhibited high success rates (teeth 82.7%).[69] Various commercial kits are available such as AmpFSTR Profiler Kit, AmpFSTR Profiler Plus Kit, the AmpFSTR COfiler Kit, and the PowerPlex 16 system are very sensitive multiplex STR amplification systems, which can be successfully used to obtain a multilocus STR profile from old teeth and bone samples with minimal amounts of human DNA or even with no detectable human DNA.[70]

### 9.3. Mitochondrial DNA (mtDNA) analysis

Long intervals between the time of death and examination of tissues complicate the genetic identification and sometimes only bone and teeth may be available for analysis. Several investigators have described regarding isolation of nuclear DNA from these materials, but all have concluded that the DNA is significantly degraded.[71] Teeth provide an excellent source for high molecular weight mtDNA that can be very valuable in forensic investigations of decomposed human remains.[72]

Mitochondrial DNA sequences offer several unique advantages for the identification of human remains.[73,74] Its analysis uses DNA extracted from mitochondrion which is another organelle of the cell. Various biological samples such as hair, bones, and teeth that lack nucleated cellular material can be analyzed with mtDNA and is very useful in solving old unsolved forensic cases.[75,76] mtDNA is a powerful tool for forensic identification as it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring have the same mitochondrial DNA as their mothers since the mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by father's sperm. In investigations involving missing persons, comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique.[77,78]

#### 9.3.1. Methods of mtDNA analysis

Forensic analysis of mitochondrial D-loop sequences using Sanger sequencing or SNP detection by mini sequencing is well established. However pyrosequencing has become an important alternative as it enables comprehensive analysis and the quantification of individual mtDNAs in samples originating from more than one individual.[79] Trace amounts of DNA or extensive degradation can lead to failure of STR analysis. In these circumstances DNA typing of mitochondrial displacement loop (D-loop) region is usually the method of choice for investigators since most of the variations in mtDNA among individuals are found within the D-loop and can be useful for discriminating among unrelated individuals.[80] The HV I/HV II mtDNA linear array assay, decreases sequencing efforts substantially and thereby reducing the cost and time in forensic analysis.[81]

### 9.4. Y-Chromosome analysis

DNA-polymorphisms on the human Y chromosome are valuable tools for understanding human evolution and migration.[82,83] Y chromosome is especially useful for tracing relationships among males or for analyzing biological evidence involving multiple male contributors since Y chromosome is passed directly from father to son. Majority of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring as a haploid entity. Thus, the Y chromosome is an invaluable record of all mutations that have occurred along male lineages throughout evolution and migration. Hence Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis.[84]

#### 9.4.1. Chromosome Y specific STR

Y-chromosome STR polymorphisms are used in deficiency paternity testing cases and especially to discriminate stains in forensic investigation when a male suspect is involved. In the analysis of stains of sexual assault that comprise the mixtures of both male as well as female and where the former is at low concentration, Y-STR analysis can be particularly helpful to detect the male DNA fraction.[85–87] The haploidy and patrilineal inheritance complicates the interpretation of a Y-STR match, because male relatives share for several generations an identical Y-STR profile. Since paternal relatives tend to live in the geographic and cultural territory of their ancestors, the Y chromosome analysis has

a potential to make inferences on the population of origin of a given DNA profile.[88,89]

DNA isolated from the teeth and bones of World War II victim's skeletal remains was used for identification through combined Y chromosome (STR) and MiniSTR approach.[90,91] Y STR is also been reported to be used in identification of human remains found in mass graves in Croatia, Bosnia and Herzegovina.[92]

#### 9.4.2. Commercial kits of Y STR analysis

The Y-PLEX 12 system enables simultaneous amplification of eleven polymorphic short tandem repeat (STR) loci, residing on the Y chromosome and amelogenin which can be used in human forensic and male lineage identification cases. Amelogenin provides results for gender identification and serves as internal control for PCR.[93]

### 9.5. X-chromosome STR

Chromosome X specific STR is used in the identification and the genomic studies of various ethnic groups in the World.[94–96] Since the size of X-chromosome STR alleles is small, generally including 100-350 nucleotides, it is relatively easy to be amplified and detected with high sensitivity.[97] X-chromosome STR (X-STR) markers are a powerful complimentary system especially in deficiency paternity testing. Many X-linked microsatellites have been evaluated but further studies are required to determine population specific statistics.[98]

### 9.6. Single nucleotide polymorphism (SNPs)

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide (A,T,C,or G) in the genome sequence is altered. For example an SNP might change the DNA sequence A**A**GGCTAA to ATGGCTAA.[99] SNPs are emerging as new markers of interest to the forensic medicine because of their small amplicon size which is useful in analysing degraded samples, lower mutation rate compared with STRs, amenable to high throughout analysis (automation), abundant in the human genome, can provide specific information about ancestry, lineage, evolution, or phenotype, and also determine sex.[100,101] The SNPs for forensic analysis can be divided into four categories such as identity-testing SNPs, lineage informative SNPs, ancestry informative SNPs, and phenotype informative SNPs.[102] In recent years, SNPs have become more widely used in a variety of applications, including medical diagnostics, population genetics, and human identity testing.[103,104] When all the DNA typing systems have failed to identify an individual, SNPs have the ability to provide definite solution and this was proved when forensic scientists used SNP technology successfully to identify several 9/11 victims.[105]

Recently Pakstis AJ et al[106] have developed a globally applicable resource of 92 SNPs for individual identification (IISNPs) with extremely low probabilities of any two unrelated individuals from anywhere in the world having identical genotypes.

Limitations of SNPs include such as no widely established core loci, and requirement of large multiplexing assays. SNPs are not likely to replace core STRs currently used in national DNA databases, linkage, substructure due to low mutation rate, multiple typing platforms which make universal SNP selection difficult.[100] Efforts are being made to investigate whether it can replace STR but nevertheless SNPs are the DNA technology of the future.

### 9.7. Gender typing

The enamel proteins that are required for the development of normal tooth enamel is encoded by the amelogenin genes. These genes are part of a small group of genes that are active on both sex chromosomes.[107] The amelogenin gene is a single copy gene, homologues of which are located on Xp22.1-Xp22.3 and Yp 11.2.[108] The variation of length in the X–Y homologous amelogenin gene (AMELX and AMELY), are used for gender identification and has become an integral part of most PCR multiplex kits employed for DNA profiling at present.[109,110]

Dental pulp is a valuable source of DNA for sex determination.[111] Komuro T et al[112] have identified the sex from the dental pulp DNA through the analysis of the peaks of X and Y locus by capillary gel electorphoresis (CGE). Many commercial kits are available like geneprint sex determination which provide better results.[113]

## 10. Conclusion

The application of DNA technology has revolutionized forensic identification procedures since its advent 25 years back. Teeth provide an excellent source of quality DNA compared to other parts of the body and has to be considered in all the forensic investigations. There is a great progress in the field of DNA research from just understanding the repeat sequence of the base pairs to predicting the physical characteristics, geographical origin and sex determination. There is an enormous DNA data being maintained in many countries which facilitates matching and identification. The use of DNA technology has also given rise to many ethical and legal problems which has to be addressed efficiently but nevertheless the field is developing at fast pace to reach new frontiers and solve many riddles hidden in the human genome.

*Conflict of interest*

None declared.

## References

1. Nuzzolese E, Di Vella G. Future project concerning mass disaster management: a forensic odontology prospectus. *Int Dent J* 2007;**57**:261–6.
2. Olaisen B, Stenersen M, Mevåg B. Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. *Nat Genet* 1997; **15**:402–5.
3. Pretty IA. Forensic dentistry: 1. Identification of human remains. *Dent Update* 2007;**34**:621–2. 624-6, 629-30.
4. Sarode SC, Zarkar GA, Kulkarni MA, Desai R. Role of forensic odontology in the world's major mass disasters: facts and figures. *SADJ* 2009;**64**:388–90. 392-3.
5. Chandra Shekar BR, Reddy CV. Role of dentist in person identification. *Indian J Dent Res* 2009;**20**:356–60.
6. Schwartz TR, Schwartz EA, Mieszerski L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental conditions. *J Forensic Sci* 1991;**36**:979–90.
7. Malaver PC, Yunis JJ. Different dental tissues as source of DNA for human identification in forensic cases. *Croat Med J* 2003;**44**:306–9.
8. Sweet D, Hildebrand D. Recovery of DNA from human teeth by cryogenic grinding. *J Forensic Sci* 1998;**43**:1199–202.
9. Pretty IA, Sweet D. A look at forensic dentistry- Part 1: the role of teeth in the determination of human identity. *Br Dent J* 2001;**190**:359–66.
10. Sweet D, DiZinno JA. Personal identification through dental evidence-tooth fragments to DNA. *J Calif Dent Assoc* 1996;**24**:35–42.
11. Sajantila A, Budowle B. Identification of individuals with DNA testing. *Ann Med* 1991;**23**:637–42.
12. Ohira H, Yamamuro Y, Kitagawa Y, Nakagawa K, Yamamoto I, Yamada Y. Effective appropriate use of dental remains and forensic DNA testing for personal identity confirmation. *Leg Med (Tokyo)* 2009;**11**(Suppl. 1):S560–2.
13. Launching forensic science International daughter journal in 2007. *Forensic Sci Int Genet* 2007;**1**:1–2.
14. Da Silva RH, Sales-Peres A, de Oliveira RN, de Oliveira FT, Sales-Peres SH. Use of DNA technology in forensic dentistry. *J Appl Oral Sci* 2007;**15**:156–61.
15. Frumkin D, Wasserstrom A, Davidson A, Grafit A. Authentication of forensic DNA samples. *Forensic Sci Int Genet* 2010;**4**:95–103.
16. Butler JM. *Forensic DNA typing biology, technology, and genetics of STR markers*. 2 nd Edition. London: Elsevier Academic Press; 2005.
17. Lee HC, Ladd C, Bourke MT, Pagliaro E, Tirnady F. DNA typing in forensic science. I. Theory and background. *Am J Forensic Med Pathol* 1994;**15**:269–82.
18. Watson JD, Crick FHC. Molecular structure of Nucleic acids. *Nature* 1953; **171**:737–8.

19. The discovery of the molecular structure of DNA - The double Helix. Nobelprize.org. (cited on 18 Jun 2010) available from: http://nobelprize.org/educational/medicine/dna_double_helix/readmore.html.
20. Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. *Nature* 1985;**316**:76–9.
21. Jeffreys AJ, Wilson V, Thein L. Hypervariable 'minisatellite' regions in human DNA. *Nature* 1985;**314**:67–73.
22. Jeffreys AJ, Wilson V, Thein SL, Weatherall DJ, Ponder BA. DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees. *Am J Hum Genet* 1986;**39**:11–24.
23. Jeffreys AJ, Brookfield JF, Semeonoff R. Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 1985;**317**:818–9.
24. Buckleton John, Triggs Christopher M, Walsh Simon J. *Forensic DNA evidence interpretation*. Washington, D.C: CRC Press; 2005.
25. Rohland N, Hofreiter M. Ancient DNA extraction from bones and teeth. *Nat Protoc* 2007;**2**:1756–62.
26. Marjanović D, Durmić-Pasić A, Bakal N, Haverić S, Kalamujić B, Kovacević L, et al. DNA identification of skeletal remains from the World War II mass graves uncovered in Slovenia. *Croat Med J* 2007;**48**:513–9.
27. Alvarez GA, Muñoz I, Pestoni C, Lareu MV, Rodríguez-Calvo MS, Carracedo A. Effect of environmental factors on PCR-DNA analysis from dental pulp. *Int J Leg Med* 1996;**109**:125–9.
28. Shiroma CY, Fielding CG, Lewis Jr JA, Gleisner MR, Dunn KN. A minimally destructive technique for sampling dentin powder for mitochondrial DNA testing. *J Forensic Sci* 2004;**49**:791–5.
29. Sweet DJ, Sweet CH. DNA analysis of dental pulp to link incinerated remains of homicide victim to crime scene. *J Forensic Sci* 1995;**40**:310–4.
30. Gaytmenn R, Sweet D. Quantification of forensic DNA from various regions of human teeth. *J Forensic Sci* 2003;**48**:622–5.
31. Lijnen I, Willems G. DNA research in forensic dentistry. *Methods Find Exp Clin Pharmacol* 2001;**23**:511–5.
32. Corte-Real A, Andrade L, Anjos MJ, Carvalho M, Vide MC, Corte-Real F, et al. The DNA extraction from the pulp dentine complex of both with and without carious. *Int Congress Ser* 2006;**1288**:710–2.
33. Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. *J Forensic Sci* 1993; **38**:1194–209.
34. Trivedi R, Chattopadhyay P, Kashyap VK. A New improved method for extraction of DNA from teeth for the analysis of hypervariable loci. *Am J Forensic Med Pathol* 2002;**23**:191–6.
35. PreseEki Z, BrkiE H, Primorac D, Drmic I. Methods of preparing the tooth for DNA isolation. *Acta Stomatol Croat* 2000;**34**:21–4.
36. Pinchi V, Torricelli F, Nutini AL, Conti M, Iozzi S, Norelli GA. Techniques of dental DNA extraction: some operative experiences. Forensic Sci Int [article in press]
37. Carracedo A. *Methods in molecular biology*. *Forensic DNA typing protocols*, vol. 297. New Jersey: Humana Press; 2005.
38. DNA purification. Protocols and applications guide. Cited on 2010 June 29 available from: http://www.promega.com/paguide/chap9a4.pdf.
39. Davoren J, Vanek D, Konjhodzić R, Crews J, Huffine E, Parsons TJ. Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves. *Croat Med J* 2007;**48**:478–85.
40. Yang DY, Eng B, Waye JS, Dudar JC, Saunders SR. Technical note: improved DNA extraction from ancient bones using silica-based spin columns. *Am J Phys Anthropol* 1998;**105**:539–43.
41. Rohland N, Hofreiter M. Comparison and optimization of ancient DNA extraction. *Biotechniques* 2007;**42**:343–52.
42. Kim K, Kim KY, Jeon E, Togloom A, Cho YO, Lee MS, et al. improved ancient DNA purification for PCR using ion-exchange columns. *Am J Phys Anthropol* 2008;**136**:114–21. Technical note.
43. Seo SB, Zhang A, Kim HY, Yi JA, Lee HY, Shin DH, et al. Technical note: Efficiency of total demineralization and ion-exchange column for DNA extraction from bone. *Am J Phys Anthropol* 2010;**141**:158–62.
44. Lee HY, Park MJ, Kim NY, Sim JE, Yang WI, Shin KJ. Simple and highly effective DNA extraction methods from old skeletal remains using silica columns. *Forensic Sci Int Genet*; 2010 [Epub ahead of print].
45. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991;**10**:506–13.
46. Tsuchimochi T, Iwasa M, Maeno Y, Koyama H, Inoue H, Isobe I, et al. Chelating resin-based extraction of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal alphoid repeat and short tandem repeats. *Am J Forensic Med Pathol* 2002;**23**:268–71.
47. Brevnov MG, Pawar HS, Mundt J, Calandro LM, Furtado MR, Shewale JG. Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. *J Forensic Sci* 2009;**54**:599–607.
48. PrepFiler forensic DNA extraction kit. Product Bulletin human identification. Applied biosystems. Cited on 2010 June 30 available from: http://marketing.appliedbiosystems.com/images/Product_Microsites/PrepFiler/PDF/54395_DNA_PrepFiler_PB_R7b-Final.pdf.
49. Polymerase chain reaction. *Molecular station* (Cited on 2010 JUL 27) available from, http://www.molecularstation.com/pcr/history-of-pcr/; 2006.
50. Bartlett JM, Stirling D. A short History of the polymerase chain reaction. *Methods Mol Biol* 2003;**226**:3–6.
51. Shampo MA, Kyle RA, Kary B. Mullis–Nobel Laureate for procedure to replicate DNA. *Mayo Clin Proc* 2002;**77**:606.
52. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K, et al. The real-time polymerase chain reaction. *Mol Aspects Med* 2006;**27**:95–125.
53. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnol (N Y)* 1992;**10**:413–7.
54. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Biotechnol (N Y)* 1993; **11**:1026–30.
55. Real time and Quantitative PCR, molecular Station. ( Cited on 2010 JUL 27) available from: http://www.molecularstation.com/real-time-pcr/.
56. Alenizi MA, Goodwin W, Hadi S, Alenizi HH, Altamar KA, Alsikel MS. Concordance between the AmpFlSTR MiniFiler and AmpFlSTR identifiler PCR amplification kits in the Kuwaiti population. *J Forensic Sci* 2009;**54**:350–2.
57. Benecke M. DNA typing in forensic medicine and in criminal investigations: a current survey. *Naturwissenschaften* 1997;**84**:181–8.
58. Zehner R, Zimmermann S, Mebs D. RFLP and sequence analysis of the cytochrome b gene of selected animals and man: methodology and forensic application. *Int J Leg Med* 1998;**111**:323–7.
59. Chakraborty R, Stivers DN, Su B, Zhong Y, Budowle B. The utility of short tandem repeat loci beyond human identification: implications for development of new DNA typing systems. *Electrophoresis* 1999;**20**:1682–96.
60. Butler JM. Genetics and genomics of core short tandem repeat loci used in human identity testing. *J Forensic Sci* 2006;**51**:253–65.
61. Urquhart A, Kimpton CP, Downes TJ, Gill P. Variation in short tandem repeat sequences–a survey of twelve microsatellite loci for use as forensic identification markers. *Int J Leg Med* 1994;**107**:13–20.
62. Ruitberg CM, Reeder DJ, Butler JM. STRBase: a short tandem repeat DNA database for human identity testing community. *Nucleic Acid Res* 2001;**29**:320–2.
63. Combined DNA index system ( CODIS). Federal Beaureau of investigations ( US ). ( cited on 2010 July 28 ) available from: http://www.justice.gov/oig/reports/FBI/a0126/final.pdf.
64. Combined DNA Index system. DNA Initiative. ( Cited on 2010 Jul 28 ) Available from: http://www.dna.gov/dna-databases/codis.
65. Boljuncić J. DNA analysis of early mediaeval individuals from the Zvonimirovo burial site in Northern Croatia: investigation of kinship relationships by using multiplex system amplification for short tandem repeat loci. *Croat Med J* 2007;**48**:536–46.
66. Clayton TM, Whitaker JP, Maguire CN. Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. *Forensic Sci Int* 1995;**76**:7–15.
67. Ricaut FX, Kolodesnikov S, Keyser-Tracqui C, Alekseev AN, Crubézy E, Ludes B. Genetic analysis of human remains found in two eighteenth century Yakut graves at At-Dabaan. *Int J Leg Med* 2004;**118**:24–31.
68. Ricaut FX, Keyser-Tracqui C, Crubézy E, Ludes B. STR-genotyping from human medieval tooth and bone samples. *Forensic Sci Int* 2005;**151**:31–5.
69. Milos A, Selmanović A, Smajlović L, Huel RL, Katzmarzyk C, Rizvić A, et al. Success rates of nuclear short tandem repeat typing from different skeletal elements. *Croat Med J* 2007;**48**:486–93.
70. Alonso A, Andelinović S, Martín P, Sutlović D, Erceg I, Huffine E, et al. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. *Croat Med J* 2001;**42**:260–6.
71. Bender K, Schneider PM, Rittner C. Application of mtDNA sequence analysis in forensic casework for the identification of human remains. *Forensic Sci Int* 2000;**113**:103–7.
72. Ginther C, Issel-Tarver L, King MC. Identifying individuals by sequencing mitochondrial DNA from teeth. *Nat Genet* 1992;**2**:135–8.
73. Koyama H, Iwasa M, Ohtani S, Ohira H, Tsuchimochi T, Maeno Y, et al. Personal identification from human remains by mitochondrial DNA sequencing. *Am J Forensic Med Pathol* 2002;**23**:272–6.
74. Yamada Y, Ohira H, Iwase H, Takatori T, Nagao M, Ohtani S. Sequencing mitochondrial DNA from a tooth and application to forensic odontology. *J Forensic Odontostomatol* 1997;**15**:13–6.
75. Ananymous. Forensic identification using mitochondrial DNA. Pyrosequencing.com. ( Cited on 2010 Jul 30) available from: www.einstein.yu.edu/dna/Pyrosequencing/./AN211_Forensic_may02.pdf.
76. Tully G, Bär W, Brinkmann B, Carracedo A, Gill P. Morling N et.al. Considerations by the European DNA profiling (EDNAP) group on the working practices, nomenclature and interpretation of mitochondrial DNA profiles. *Forensic Sci Int* 2001;**124**:83–91.
77. Budowle B, DiZinno JA, Wilson MR. Interpretation Guidelines for mitochondrial Dna sequencing. http://www.promega.com/. (Cited on 2010 Jul 30 ) available from: http://www.promega.com/geneticidproc/ussymp10proc/37budowle.pdf.
78. Sullivan KM, Hopgood R, Gill P. Identification of human remains by amplification and automated sequencing of mitochondrial DNA. *Int J Leg Med* 1992;**105**:83–6.
79. Andréasson H, Asp A, Alderborn A, Gyllensten U, Allen M. Mitochondrial sequence analysis for forensic identification using pyrosequencing technology. *Biotechniques* 2002;**32**:124–6.
80. Anjum GM, Du W, Klein R, Amara U, Huber-Lang M. Schneider EMetal. Pyrosequencing-based strategy for a successful SNP detection in two hypervariable regions: HV-I/HV-II of the human mitochondrial displacement loop. *Electrophoresis* 2010;**31**:309–14.

81. Divne AM, Nilsson M, Calloway C, Reynolds R, Erlich H, Allen M. Forensic casework analysis using the HVI/HVII mtDNA linear array assay. *J Forensic Sci* 2005;**50**:548–54.
82. Zhong H, Shi H, Qi XB, Xiao CJ, Jin L, Ma RZ, et al. Global distribution of Y-chromosome haplogroup C reveals the prehistoric migration routes of African exodus and early settlement in East Asia. *J Hum Genet* 2010;**55**:428–35.
83. Su B, Xiao J, Underhill P, Deka R, Zhang W, Akey J, et al. Y-Chromosome evidence for a northward migration of modern humans into Eastern Asia during the last Ice Age. *Am J Hum Genet* 1999;**65**:1718–24.
84. Krausz C, Quintana-Murci L, Forti G. Y chromosome polymorphisms in medicine. *Ann Med* 2004;**36**:573–83.
85. Jobling MA, Pandya A, Tyler-Smith C. The Y chromosome in forensic analysis and paternity testing. *Int J Leg Med* 1997;**110**:118–24.
86. Henke J, Henke L, Chatthopadhyay P, Kayser M, Dülmer M, Cleef S, et al. Application of Y-chromosomal STR haplotypes to forensic genetics. *Croat Med J* 2001;**42**:292–7.
87. Iida R, Kishi K. Identification, characterization and forensic application of novel Y-STRs. *Leg Med (Tokyo)* 2005;**7**:255–8.
88. Gusmão L, Butler JM, Carracedo A, Gill P, Kayser M, Mayr WR, et al. DNA Commission of the International Society of Forensic Genetics (ISFG): an update of the recommendations on the use of Y-STRs in forensic analysis. *Forensic Sci Int* 2006;**157**:187–97.
89. Roewer LY. chromosome STR typing in crime casework. *Forensic Sci Med Pathol* 2009;**5**:77–84.
90. Marjanović D, Durmić-Pasić A, Kovacević L, Avdić J, Dzehverović M, Haverić S, et al. Identification of skeletal remains of Communist Armed Forces victims during and after World War II: combined Y-chromosome (STR) and MiniSTR approach. *Croat Med J* 2009;**50**:296–304.
91. Definis GM, Sutlović D. Skeletal remains from World War II mass grave: from discovery to identification. *Croat Med J* 2007;**48**:520–7.
92. Andelinović S, Sutlović D, Erceg Ivkosić I, Skaro V, Ivkosić A, Paić F, et al. Twelve-year experience in identification of skeletal remains from mass graves. *Croat Med J* 2005;**46**:530–9.
93. Shewale JG, Nasir H, Schneida E, Gross AM, Budowle B, Sinha SK. Y-chromosome STR system, Y-PLEX 12, for forensic casework: development and validation. *J Forensic Sci* 2004;**49**:1278–90.
94. Kang L, Li S. X-chromosome STR polymorphism of Luoba ethnic group living in Tibet (SW China). *Forensic Sci Int* 2006;**156**:88–90.
95. Yu B, Zhang H, Li S. X-chromosome STRs polymorphisms of Han ethnic group from Northwest China. *Forensic Sci Int* 2005;**153**:269–71.
96. Aler M, Sánchez-Diz P, Gomes I, Gisbert M, Carracedo A, Amorim A, et al. Genetic data of 10 X-STRs in a Spanish population sample. *Forensic Sci Int* 2007;**173**:193–6.
97. Gu S, Li S. X-chromosome STRs analysis of Ewenke ethnic population. *Forensic Sci Int* 2006;**158**:72–5.
98. Tariq MA, Ullah O, Riazuddin SA, Riazuddin S. Allele frequency distribution of 13 X-chromosomal STR loci in Pakistani population. *Int J Leg Med* 2008; **122**:525–8.
99. SNP Fact Sheet. Human genome project. U.S. Department of Energy genome Program's biological and environmental research information system (BERIS). (Cited on 2010 July 28 ) available from: http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml.
100. Butler JM, Budowle B, Gill P, Kidd KK, Phillips C, Schneider PM, et al. Report on ISFG SNP panel Discussion. *Forensic Sci Int* 2008;(suppl 1):471–2.
101. Kim JJ, Han BG, Lee HI, Yoo HW, Lee JK. Development of SNP-based human identification system. *Int J Leg Med* 2010;**124**:125–31.
102. Budowle B, van Daal A. Forensically relevant SNP classes. *Biotechniques* 2008;**44**:603–8. 610.
103. Sobrino B, Carracedo A. SNP typing in forensic genetics: a review. *Methods Mol Biol* 2005;**297**:107–26.
104. Sobrino B, Lareu M, Brion M, Carracedo A. SNP genotyping with single base extension-tag microarrays. *Int Congress Ser* 2004;**1261**:331–3.
105. Gill JR. 9/11 and the New York City Office of Chief medical Examiner. *Forensic Sci Med Pathol* 2006;**2**:29–32.
106. Pakstis AJ, Speed WC, Fang R, Hyland FC, Furtado MR, Kidd JR, et al. SNPs for a universal individual identification panel. *Hum Genet* 2010;**127**:315–24.
107. Chen E, Yuan ZA, Collier PM, Greene SR, Abrams WR, Gibson CW. Comparison of upstream regions of X- and Y-chromosomal amelogenin genes. *Gene* 1998;**216**:131–7.
108. Sasaki S, Shimokawa H. The amelogenin gene. *Int J Dev Biol* 1995;**39**:127–33.
109. Mitchell RJ, Kreskas M, Baxter E, Buffalino L, Van Oorschot RA. An investigation of sequence deletions of amelogenin (AMELY), a Y-chromosome locus commonly used for gender determination. *Ann Hum Biol* 2006; **33**:227–40.
110. Tzvetkov MV, Meineke I, Sehrt D, Vormfelde SV, Brockmöller J. Amelogenin-based sex identification as a strategy to control the identity of DNA samples in genetic association studies. *Pharmacogenomics* 2010;**11**:449–57.
111. Murakami H, Yamamoto Y, Yoshitome K, Ono T, Okamoto O, Shigeta Y, et al. Forensic study of sex determination using PCR on teeth samples. *Acta Med Okayama* 2000;**54**:21–32.
112. Komuro T, Nakamura M, Tsutsumi H, Mukoyama R. Gender determination from dental pulp by using capillary gel electrophoresis of amelogenin locus. *J Forensic Odontostomatol* 1998;**16**:23–6.
113. Pouchkarev VP, Shved EF, Novikov PI. Sex determination of forensic samples by polymerase chain reaction of the amelogenin gene and analysis by capillary electrophoresis with polymer matrix. *Electrophoresis* 1998;**19**:76–9.